

The University of Chicago

STUDIES ON THE RÔLE OF THE
SPLEEN IN EXPERIMENTAL
POLIOMYELITIS

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY

1935

By
EDWIN H. LENNETTE

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STUDIES ON THE RÔLE OF THE SPLEEN IN EXPERIMENTAL POLIOMYELITIS*

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The cellular aspects of immunity to poliomyelitis, almost completely overshadowed by the intensive investigations conducted on its humoral features, have received far less attention than they merit. Leiner and von Wiesner (1) were unable to detect poliomyelitis virus in the spleen, and Levaditi (2) states that the virus is not found in the kidney, bone marrow, liver or spleen. Flexner and Amoss (3), however, found that the virus survived in the spleen for at least 17 days after intravenous inoculation, but that its "virulence" had been diminished. Brebner (4) stressed the fact that many intracerebral lethal doses of virus can be injected directly into the spleens of normal monkeys with little danger of infection resulting, but that similar injections into immune monkeys are followed by a severe, possibly anaphylactic, reaction and death (5).

The experimental work reported here is concerned with the effect of splenectomy on resistance to poliomyelitis and with the rôle of the spleen in fixing and disposing of the virus. *Rhesus* monkeys (*Macaca mulatta*) were used in all the experiments.

Effect of Splenectomy on Resistance to Infection

The functional rôle of the spleen in resistance to infection has been the subject of numerous investigations; as a result of these studies it is generally accepted that this organ is intimately associated with immune states, natural or acquired. Most investigations, however, have been concerned with bacteria or other large

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forms, such as the blood protozoa, the filterable viruses receiving little consideration.

The importance of the spleen in an infection due to a highly neurotropic virus such as that of poliomyelitis, which apparently follows a direct course from the nasal mucosa to the central nervous system *via* the olfactory nerves (6), depends a great deal on whether the virus remains confined to its neural pathways or whether it circulates in the blood at any period during the pathogenesis of the disease. Since attempts to demonstrate poliomyelitis virus in the blood stream following its intracerebral or intranasal administration have seldom been successful (7), and since introduction of the virus by either of these routes is practically always followed by the clinical disease, we resorted to intravascular inoculation, which is effective only when large amounts of virus are injected.

Methods

Test monkeys were splenectomized under deep ether or nembutal anesthesia 2 to 3 weeks prior to inoculation, so that the inflammatory reaction about the incision would have an opportunity to subside and healing occur, thus preventing possible infection of local nerve tissue. Splenectomized convalescent or immunized monkeys and non-splenectomized normal monkeys were used as controls.

The MV strain of virus was used in all the experiments here reported, the inoculum being prepared by grinding pooled, glycerolated poliomyelitic spinal cords with sufficient physiological salt solution to give a 10 per cent suspension, which was then centrifugated at 2,000 R.P.M. for 30 minutes. The markedly turbid supernatant was drawn off, filtered through paper and injected intravenously; inoculation was done very slowly through a fine needle, since such preparations were found to be quite toxic and occasionally caused an anaphylaxis-like reaction with death.

When large amounts of virus (2 or 3 daily injections, see Table I) were given intravenously to non-splenectomized control monkeys, half the animals (9 of 18) became infected, while the same proportion of splenectomized monkeys (4 of 9) succumbed when given similar treatment. None of 14 immunized or convalescent animals without spleens reacted to these large doses of virus. On the other hand, when smaller doses of virus (single injections, Table I) were administered to 6 normal monkeys as controls, none showed any reaction, whereas 2 of 5 monkeys previously splenec-

tomized became paralyzed.¹ This was the only evidence that splenectomy played any part in lowering the resistance of the experimental animal to intravenously injected virus.

These results suggest that when large doses of virus are used infection possibly results from an overwhelming of the defensive mechanisms of the nasal mucosa (8) or from penetration of the virus through some other barrier (9), regardless of whether or not the spleen is present, and is a chance occurrence. Conversely, small amounts of virus may fail to infect the intact animal because they are quickly fixed by the spleen and so fail to reach the central nervous system,

TABLE I
Effect of Splenectomy on Infection by the Intravenous Route

Description	Virus dosage (10 cc.)		Number of monkeys inoculated	Poliomyelitis	
	Injections	Virus		Number positive	Number negative
		<i>per cent</i>			
Splenectomized immune and convalescent monkeys	2 or 3	5 or 10	14	0	14
Splenectomized normal monkeys	2 or 3	5 or 10	9	4	5
	1	10	5	2	3
Non-splenectomized normal monkeys	2 or 3	5 or 10	18	9	9
	1	10	6	0	6

whereas in the absence of the spleen the virus may possibly circulate freely for a sufficiently long interval to reach the nervous system and initiate infection.

We next attempted to determine the effect of splenectomy subsequent to inoculation. The intravenous and intrasplenic routes of inoculation were used.

Six monkeys were given one intravenous injection of 10 cc. 10 per cent virus suspension and pairs of them splenectomized at 1, 3 and 5 day intervals after the injection. Observations and temperatures were recorded daily for 4 weeks, but no reaction to the virus was noted at any time (Table II). Splenectomy, therefore, did not

¹ In another connection, we have recently injected a series of 23 intact normal monkeys with similar single doses of virus, and in no case did paralysis ensue.

render these animals susceptible to doses of virus subinfective for non-splenectomized monkeys.

Using the intrasplenic route, essentially the same results were obtained (Table III). The spleens were exteriorized to minimize leakage of the inoculum into the peritoneal cavity or subcutaneous tissues and to avoid entering the belly twice. Exteriorizing was

TABLE II

Effect of Removing Spleen at Various Intervals after Intravenous Administration of Virus

Number of monkeys inoculated	Date of injection*	Date of splenectomy	Interval	Results
			days	
2	July 20	July 21	1	No infection
2	" 20	" 23	3	" "
2	" 20	" 25	5	" "

* 10 cc. 10 per cent virus-cord intravenously.

TABLE III

Effect of Removing Spleen at Various Intervals after Intrasplenic Administration of Virus

Monkey	Date of injection*	Date of splenectomy	Interval	Results
			days	
7	Apr. 24	Apr. 25	1	Poliomyelitis
8	" 16	" 17	1	No infection
9	" 16	" 19	3	" "
10	" 16	" 19	3	" "
11	" 16	" 21	5	" "
12	" 24	" 29	5	" "

* 2 cc. 10 per cent virus-cord intrasplenically.

accomplished by loosely suturing the abdominal muscles about the splenic pedicle and closing the skin over the spleen. Enough of the pedicle was brought out to make the subsequent splenectomy simple; the skin was incised to expose the spleen, the pedicle was clamped off, the spleen removed and bleeding vessels tied off or cauterized. Careful inspection for remnants of splenic tissue was made before closing the incision.

Six monkeys with exteriorized spleens were injected intrasplenically with 2 cc. 10 per cent virus suspension 2 weeks after operation. Splenectomy was done 1, 3 and 5 days after injection. In only one instance did infection occur; this was in a monkey splenectomized 24 hours after injection, paralysis appearing 6 days after the operation. We believe that infection in this animal was probably caused by leakage of the virus into the peritoneal cavity or subcutaneous tissues because: (a) doses of virus 5 times larger than the one used here uniformly failed to produce infection by the intravenous route, and (b) a large part of the inoculum, as will be shown later, is fixed by the spleen and so removed from the body when the spleen is removed. This experiment, therefore, did not demonstrate that splenectomy subsequent to intravenous or intrasplenic introduction of virus served to decrease resistance. This absence of increased susceptibility, however, may be due in major part to the removal of a large portion of the infectious agent from the body after its accumulation in the spleen.

Attempts to Infect Monkeys by Intrasplenic Injections of Virus

The experiments just described show that small doses of virus given intrasplenically are no more effective in producing poliomyelitis than are similar doses given intravenously. We were interested in determining the effects of repeated administrations of virus intrasplenically, since Brebner (4) and Schaeffer (10) state that many lethal intracerebral doses of virus can be given by this route without infecting the animal.

Seven monkeys with exteriorized spleens were given intrasplenic injections of virus at weekly intervals. 3 other animals were given only 2 injections with a 2 day interval between the inoculations. The results (Table IV) confirm the findings of Brebner and Schaeffer. Only 2 of the 10 animals succumbed to infection, and the possibility of virus leakage into the peritoneal cavity or subcutaneous tissues, or infection of nerve endings within the spleen, cannot be ruled out.

The administration of virus by this route leads to the appearance of humoral antibodies, 2 injections presumably being sufficient to stimulate their formation in amounts detectable by our usual serum neutralization technic (1.5 cc. of serum mixed with 0.5 cc. 1 per cent

virus, the entire amount being injected intracerebrally into test monkeys after incubation for 2 hours at 37°C. and overnight in the refrigerator).

Fixation of Virus by the Spleen

Luckhardt and Becht (11) found that antigen² injected intravenously accumulates in the spleen in considerable quantity. Topley (12), using *Bacterium paratyphosum* B, noted that the spleen re-

TABLE IV
Effect of Intrasplenic Inoculation of Poliomyelitis Virus

Monkey	Intrasplenic injection	Number of weekly injections	Results	Serum neutralization test*
13	2 cc. 10 per cent virus	1†	Poliomyelitis	0
14	1 " 20 " " "	3†	"	0
15	1 " 20 " " "	6	No infection	Positive
16	1 " 20 " " "	6	" "	"
17	1 " 20 " " "	6	" "	"
18	1 " 20 " " "	6	" "	"
19	1 " 20 " " "	6	" "	"
20	2 " 10 " " "	2‡	" "	"
21	2 " 10 " " "	2‡	" "	"
22	2 " 10 " " "	2‡	" "	Negative

0 = not done.

* Serum obtained 4 weeks after injection of virus.

† Poliomyelitis before series carried further; monkey 13 became paralyzed 7th day after injection, monkey 14 on the 4th day after the 3rd injection.

‡ Injections given 2 days apart.

moves much of the antigen from the blood and is active in destroying it. Accumulation and destruction in the spleen of malarial parasites, organisms especially suitable for studies on cellular immunity, has been described in detail recently by Taliaferro and Cannon (13). Deutsch (14) observed that splenectomy performed before inoculation of bacterial antigen exerted little or no effect on subsequent antibody production, while splenectomy done 3 to 5 days after inoculation significantly depressed antibody formation.

² Goat's red blood cells, injected into dogs.

To determine the rôle of the spleen in fixing and disposing of the virus, 18 monkeys, including immune animals as controls, were injected intravenously or intrasplenically. Single injections of virus were given in every instance; 10 cc. of a 10 per cent virus suspension intravenously, or 2 cc. of the same suspension intrasplenically. On the 1st, 3rd and 5th days of the experiment the spleens were removed aseptically under ether or nembutal anesthesia. Immediately after removal the spleens were ground with 3 volumes of sterile saline and the suspension placed in the refrigerator for several hours to allow settling of large particles; 2 cc. of the supernatant fluid was injected intracerebrally into test monkeys.

TABLE V

Accumulation of Poliomyelitis Virus in the Spleen after Intravenous Injection

Spleen of monkey	History of monkey	Interval between inoculation and splenectomy	Test monkey*	Results in test monkeys
		<i>days</i>		
A	Normal	1	23	Poliomyelitis
B	"	1	24	"
C	Convalescent	1	25	No infection
D	"	1	26	" "
E	Immune	1	27	" "
F	Normal	3	28	" "
G	"	3	29	" "
H	"	5	30	" "
J	"	5	31	" "
K	Convalescent	5	32	" "
L	Immune	5	33	" "
M	"	5	34	" "

* Each animal received intracerebrally 2.0 cc. of a 25.0 per cent crude suspension of spleen in saline.

The suspensions of spleens removed from normal monkeys 24 hours after inoculation of virus into the blood or spleen contained virus demonstrable by inoculation tests, while spleens removed on the 3rd and 5th days did not (Tables V and VI). In no instance was virus detectable in the spleens of immune animals. No attempt was made to detect virus in the blood at the time of splenectomy, since Clark, Fraser and Amoss (15) have found that the virus persists in the blood for at least 3 days when large amounts (180 cc.) are administered

intravenously, but is detected only occasionally at the end of 24 hours when small amounts (10 cc.) are given.

Virus introduced into the blood stream of normal monkeys, then, rapidly accumulated in the spleen, persisted for at least 24 hours and disappeared by the 3rd day. The failure to detect virus in immune spleens is perhaps due to its neutralization by humoral antibodies or to rapid destruction by sensitized splenic tissue (16).

To determine the fate of the virus, we tested for the presence of neutralizing antibodies in the sera of monkeys splenectomized before and at intervals after intravenous injection of virus.

TABLE VI
Fixation and Destruction of Poliomyelitis Virus by the Spleen after Intrasplenic Injection

Spleen of monkey	History of monkey	Interval between inoculation and splenectomy	Test monkey*	Results in test monkeys
		<i>days</i>		
N	Normal	1	35	Poliomyelitis
P	"	1	36	"
Q	"	3	37	No infection
R	"	3	38	" "
S	"	5	39	" "
T	"	5	40	" "

* Each monkey received 2.0 cc. intracerebrally of a 25.0 per cent crude suspension of spleen in saline.

Eight non-splenectomized normal monkeys and 2 splenectomized normal monkeys were given a single injection of 10 cc. 10 per cent virus intravenously. The splenectomized monkeys and 2 of the intact animals served as controls; the remaining 6 animals were splenectomized at 1, 3 and 5 day intervals, as shown in Table V (monkeys A, B, F, G, H and J).

All the experimental animals survived without signs of infection; sera obtained 4 to 6 weeks after inoculation were tested for antiviral properties (Table VII), 1.5 cc. of serum being tested against 0.5 cc. 1.0 per cent virus. Table VII shows that neutralizing antibodies were present in the sera of both the non-splenectomized controls and the animals splenectomized prior to inoculation. Those animals

which were splenectomized subsequent to inoculation, however, failed to develop neutralizing antibodies.

Removal of the spleen 2 weeks prior to inoculation, therefore, did not qualitatively affect antibody formation. Splenectomy within 5 days after inoculation, however, seemed to suppress antibody formation. The simplest explanation appears to be that in the absence of the spleen other parts of the reticulo-endothelial system remove the

TABLE VII

*Effect of Splenectomy on Antibody Formation after Intravenous Administration of Virus**

Mon-key	Treatment	Results	Serum neutralization test†
41	Splenectomized 14 days before inoculation	No infection	Positive
42	" 14 " " "	" "	"
A	Splenectomized 1 day after inoculation	" "	Negative
B	" 1 " " "	" "	"
F	Splenectomized 3 days after inoculation	" "	"
G	" 3 " " "	" "	"
H	Splenectomized 5 days after inoculation	" "	"
J	" 5 " " "	" "	"
43	Non-splenectomized control	" "	Positive
44	" " "	" "	"

* Each animal received one dose of 10.0 cc. 10 per cent crude virus-cord suspension.

† Serum obtained 4 to 6 weeks after inoculation.

virus from the circulation; the spleen, when present, filters out an appreciable amount, as evidenced by the fact that spleens removed 24 hours after vascular inoculation contain active virus. Inasmuch as removal of the spleen at this time leads to a simultaneous removal of antigen, little or no antibody formation might be expected. The fact that spleens removed on the 3rd and 5th days contain no demonstrable virus, and the animals from which such spleens are removed fail, in contrast with control animals, to develop antiviral bodies,

suggests that little or no virus escapes from the spleen after its fixation there, and that it is destroyed *in situ*. These findings agree with those of Topley (12) and Deutsch (14), who used bacterial antigens.

Attempts to Detect Virus in the Spleens of Poliomyelitic Monkeys

The hyperplasia of lymphoid tissue which occurs throughout the body in human and experimental poliomyelitis has been interpreted by many authors as evidence of a systemic infection (7 a), a view supported by the claims of clinicians that patients react as to a generalized infection. The virus has not been detected in human blood (7 a) even when large amounts have been tested, but has been found occasionally in monkey blood (7 a) at the height of paralysis. Failure to isolate the virus more frequently may be due to the presence of neutralizing antibodies, which have been detected in the blood of monkeys 36 hours after the onset of paralysis (17) and in human cases during and even prior to paralysis (18), but since the virus displays such great affinity for nervous tissue, one would expect to find little outside the central nervous system. According to Fairbrother and Hurst (19), aside from the central nervous system the virus is found with regularity only in the tonsils and nasopharyngeal mucosa.

In the experiments described above, the virus, when administered intravenously or intrasplenically, was demonstrated in the spleen at the end of 24 hours, but not at 72 hours. Inability to detect the virus under these conditions, as well as in the blood of human cases, and of monkeys inoculated by other routes, may rest on its destruction by phagocytes or neutralization by antibodies. The latter possibility is of fundamental importance as well as of considerable interest. The simultaneous presence of virus and antibodies in the blood has been demonstrated in experimental vaccinia by Smith (20) and in a human case of yellow fever by Berry and Kitchen (21). Discussing such observations, Topley and Wilson (22) point out that "caution should be exercised in assuming that any tissue extract or body fluid is necessarily free from living virus because it is not infectious."

To gain information on these points we prepared extracts of spleens removed from 10 monkeys in the acute stage of poliomyelitis following intracerebral

inoculation of virus. Crude suspensions were made of 5 spleens by grinding the tissue with 3 volumes of saline and freezing and thawing 10 times in a carbon dioxide-alcohol mixture. After several hours in the refrigerator to allow coarse particles to settle out, 2.0 cc. of the supernatant fluid was injected intracerebrally into test monkeys. None of these animals showed any clinical evidence of infection. 2 were subsequently inoculated intracerebrally with 1.0 cc. of 1.0 per cent crude virus-cord suspension and succumbed to poliomyelitis. Another pair was sacrificed to examine the spinal cords, which in both instances were found to show no histological evidence of poliomyelitis.

The remaining 5 spleens were perfused with physiological salt solution until the perfusate was clear and the organ acquired a pale yellow to white color. The tissue was then ground with 3 volumes of saline, frozen and thawed 10 times in a carbon dioxide-alcohol mixture and injected intracerebrally (2.0 cc.). One test animal succumbed to poliomyelitis, paralysis appearing 15 days after inoculation of the splenic extract; the other 4 gave no evidence of infection. 2 died of intercurrent infection 1 and 3 months after inoculation and 2 were sacrificed at the end of 2 months observation. In 2 of these animals the histologic findings were negative, in one the findings were compatible with a diagnosis of poliomyelitis but were not typical of the experimental disease, and in one typical poliomyelitic changes were present.

The failure to demonstrate virus in crude suspensions, in contrast to extracts of the perfused organs, may depend on the concomitant presence of antibodies. To test this hypothesis, the following experiments were done.

Experiment 1.—The spleens of 2 normal and 2 immune monkeys were removed aseptically and ground with sterile saline to give a 25 per cent suspension. After the coarser particles had settled out, 1.5 cc. of the turbid supernatant was mixed with 0.5 cc. of a 1.0 per cent crude virus-cord suspension. The mixtures were incubated 2 hours at 37°C., and overnight in the refrigerator; after being warmed to body temperature, 2.0 cc. was injected intracerebrally into monkeys. Suspensions of immune spleens neutralized the virus, while both normal spleens failed to do so (Table VIII).

Experiment 2.—Spleens were removed aseptically from 9 monkeys (3 immune, 3 normal and 3 dying of poliomyelitis) and perfused with physiological salt solution until the perfusate was clear. At the end of this treatment the spleens presented a glistening, yellow, wax-like appearance. Microscopic sections showed the presence of a relatively small number of red blood cells and also that a large part of the pulp cells had been washed out. The perfused spleens were ground with saline to make a 25 per cent suspension, the supernatant being a yellowish, heavily opalescent fluid as compared with the deep brown-red of the crude suspensions. The extracts were frozen 10 times in a carbon dioxide-alcohol mix-

ture, passed through a Berkefeld N filter and the filtrates used in neutralization tests, which were done in the same manner as for the crude suspensions above. No neutralization of the virus occurred with any of the extracts (Table VIII).

TABLE VIII
*Tests for Neutralizing Antibodies in Crude and in Perfused Extracts of
Monkey Spleens*

Test monkey	Spleen from	Intracerebral injection into test monkeys	Results in test monkeys
45	Immune monkey 1	2.0 cc. mixture of 1.5 cc. crude 25 per cent suspension of spleen plus 0.5 cc. 1 per cent virus-cord	No infection
46	" " 2		" "
47	Normal monkey 1		Poliomyelitis
48	" " 2		"
49	Poliomyelitic monkey 1*	2.0 cc. mixture of 1.5 cc. 25 per cent suspension of perfused spleen plus 0.5 cc. 1 per cent virus-cord	Poliomyelitis
50	" " 2*		"
51	" " 3*		"
52	Normal monkey 1		"
53	" " 2		"
54	" " 3		"
55	Immune monkey 1		"
56	" " 2		"
57	" " 3		"
58		2.0 cc. mixture of 1.5 cc. pooled convalescent monkey serum plus 0.5 cc. 1 per cent virus-cord	No infection
59		2.0 cc. mixture of 1.5 cc. pooled normal monkey serum plus 0.5 cc. 1 per cent virus-cord	Poliomyelitis

* Spleen removed at height of paralysis after intracerebral injection of virus.

These experiments show that it is possible to remove humoral antibodies from the spleen and suggest that our success in detecting virus in the perfused spleens was due to the removal of a neutralizing substance. If virus and neutralizing substances are present simultane-

ously in poliomyelitic spleens the virus might, if present in sufficiently high concentration, act as an antigen in stimulating the formation of poliocidal antibodies, since several authors (23) have reported a high incidence of immunity in monkeys following injections of neutralized mixtures of virus and serum.

Spleens were removed aseptically from monkeys at the height of paralysis following intracerebral inoculation of virus. 25 per cent suspensions in saline were prepared from each spleen and injected into 3 monkeys by various routes. Injections were given at the rate of 2 a week for 7 weeks, a total of 41.0 cc. being administered to each monkey. Sera obtained 6 weeks after the last injection failed to neutralize the test virus (1.5 cc. serum plus 0.5 cc. 1.0 per cent crude virus-cord suspension).

Considering the results obtained in the perfusion experiments, it is unlikely that all the spleens were devoid of virus. It appears more probable that the relatively large amount of inoculum contained too little virus to excite an appreciable antibody response. Although poliomyelitic spleens may contain amounts of virus infectious upon intracerebral inoculation it does not necessarily follow that this concentration is sufficient, in view of the known weak antigenic properties of the virus, to elicit any marked antibody response. This point has been emphasized recently by Kramer (23 *d*) who found that neutralized mixtures containing the equivalent of 1.5 gm. of virus-cord failed in many instances to produce sufficient antibodies to neutralize a small test dose of virus.

DISCUSSION

The necessity of using huge doses of virus to produce experimental poliomyelitis in *Macaca mulatta* by the intravenous route is well known and generally attributed to the difficulty encountered by the virus in reaching susceptible nerve tissue, a difficulty readily appreciated when it is considered that the nervous system is well isolated from other tissues (24). This insulating mechanism, however, is defective in at least one region, *viz.* the olfactory mucosa, where the endings of the first and thirteenth cranial nerves lie free. Lennette and Hudson (8) have shown that following intravenous administration of large doses the virus can be detected in nasal washings and that infection probably depends to a considerable degree on the amount

of virus excreted onto the nasal mucosa, a concept which finds support in the recent demonstration (25) of lesions in the olfactory bulbs of monkeys succumbing to infection by this route and in the ability of nasally instilled picric acid to prevent infection after vascular inoculation of the virus (26). While large amounts of virus accumulate in the spleen, and therefore should decrease the amount available for excretion onto the nasal mucosa, we were unable to detect any difference in susceptibility to infection, after repeated intravascular inoculation, between splenectomized and non-splenectomized animals. The possibility that the large doses of virus used (3 daily injections of 10 cc. 10 per cent crude virus-cord suspension) obscured any slight differences in susceptibility was considered, and the dosage decreased. A single injection of crude 10 per cent virus, while found not to be infective for a large group of non-splenectomized monkeys proved to be so for a small number of splenectomized animals. The results, while not conclusive due to the small number of splenectomized animals employed, suggest that some decrease in resistance to infection may occur when the animal is splenectomized prior to inoculation.

Splenectomy subsequent to intravenous or intrasplenic injection of virus did not appear to alter resistance to infection by these routes; this may be due, however, to simultaneous removal of much of the virus, which was found to accumulate rapidly in the spleen. Spleens removed from animals inoculated by either of the routes mentioned contained virus 24 hours after inoculation, but none after 3 or 5 days. The results of these experiments also suggest that splenectomy, with a simultaneous removal of a considerable portion of the antigen, interferes with antibody formation. None of 6 monkeys splenectomized after vascular inoculation developed neutralizing antibodies whereas the controls did so. Also, since no virus was detected in spleens removed 3 and 5 days after inoculation, and since the animals from which these spleens were obtained failed to develop poliocidal antibodies, we are inclined to believe that the virus is destroyed in the spleen.

Attempts to detect virus in the spleens of monkeys infected by routes other than the vascular were complicated by the presence of a neutralizing factor. Crude suspensions of poliomyelitic spleens failed to produce poliomyelitis on intracerebral injection, while similar

spleens after perfusion in one case caused frank paralysis and in two others lesions in the spinal cord. In one instance the lesions were typical of the experimental disease and in the other compatible with a diagnosis of poliomyelitis, although not absolutely typical.

An attempt to immunize test animals with poliomyelitic spleen tissue failed. The negative results presumably were due to a small content of virus rather than to the presence of neutralizing antibodies.

SUMMARY AND CONCLUSIONS

1. No difference in susceptibility to infection between splenectomized and non-splenectomized monkeys was observed when virus was injected intravenously in large amounts.

2. In a small series of animals, the results suggested that splenectomy decreased resistance to amounts of virus subinfective for non-splenectomized monkeys.

3. Splenectomy subsequent to intravenous or intrasplenic inoculation of amounts of virus subinfective for non-splenectomized monkeys did not render the animals susceptible.

4. Following vascular injection, the virus accumulates in the spleen, where it can be detected 24 hours after inoculation, but not at 3 or 5 days.

5. Evidence that splenectomy interferes with antibody formation is presented and discussed.

6. We were unable to demonstrate virus in crude extracts of poliomyelitic spleens but were successful with perfused spleen extracts. This may be due to removal of a neutralizing factor.

7. Attempts to immunize test animals with virus present in poliomyelitic spleens were negative.

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